(h) *Samples and protocols.* For each lot of vaccine, the following material shall be submitted to the Director, Center for Biologics Evaluation and Research, Food and Drug Administration, 8800 Rockville Pike, Bethesda, MD 20892.

(1) A sample of no less than 20 milliliters of the final product for pertussis vaccine testing.

(2) Protocols showing summaries of the manufacturing processes and the results of all mouse toxicity (§ 620.5) and potency (§ 620.4) tests performed.

[38 FR 32064, Nov. 20, 1973, as amended at 41 FR 35480, Aug. 23, 1976; 48 FR 13025, Mar. 29, 1983; 49 FR 23834, June 8, 1984; 51 FR 15610, Apr. 25, 1986; 55 FR 11013, Mar. 26, 1990]

Subpart B—Typhoid Vaccine

§ 620.10 Typhoid Vaccine.

The proper name of this product shall be Typhoid Vaccine which shall be an aqueous or dried preparation of killed *Salmonella typhi* bacteria.

[48 FR 7167, Feb. 18, 1983]

§ 620.11 Production.

(a) *Strain of bacteria.* (1) Strain Ty 2 of *Salmonella typhi* shall be used in the manufacture of Typhoid Vaccine.

(2) The antigenic integrity of the Ty 2 strain shall be verified by an appropriate serological procedure.

(b) *Propagation of bacteria.* The culture medium for propagation of *S. typhi* shall not contain ingredients known to be capable of producing allergenic effects in human subjects. The harvested bacteria shall be free of extraneous bacteria, fungi, and yeasts, as demonstrated by microscopic examination and cultural methods.

(c) *Bacterial content.* (1) The number of bacteria in the concentrate of harvested bacteria shall be estimated not later than 2 weeks after harvest and before any treatment capable of altering the accuracy of the estimate.

(2) The number of *S. typhi* bacteria in the vaccine shall not exceed 10^9 per milliliter.

(d) *Nitrogen content.* The total nitrogen content of the vaccine shall not exceed 0.035 mg./ml. for nonextracted bacteria preparations and shall not exceed 0.023 mg./ml. for acetone-extracted bacteria preparations.

(e) *Preservative.* Aqueous vaccine and the solution for reconstitution supplied with dried vaccine shall contain a preservative. Dried vaccine shall not contain a preservative.

[38 FR 32064, Nov. 20, 1973, as amended at 48 FR 7167, Feb. 18, 1983]

§ 620.12 U.S. Standard preparations.

The following U.S. Standard preparations shall be obtained from the Center for Biologics Evaluation and Research (HFB–210), Food and Drug Administration, 8800 Rockville Pike, Bethesda, MD 20892, for use as prescribed in this part:

(a) *Vaccine standard.* The U.S. Standard Typhoid Vaccine for determining the potency of Typhoid Vaccine.

(b) *Opacity standard.* The U.S. Opacity Standard for adjusting the opacity of the suspension from which the challenge culture is prepared.

[48 FR 7167, Feb. 18, 1983, as amended at 49 FR 23834, June 8, 1984; 51 FR 15610, Apr. 25, 1986; 55 FR 11015, Mar. 26, 1990]

§ 620.13 Potency test.

The number of potency units per milliliter shall be estimated for each lot of vaccine from the results of simultaneous mouse protection tests of the vaccine under test and of the U.S. Standard Typhoid Vaccine. At least four dilutions of each lot of vaccine shall be tested. The test shall be performed as follows:

(a) *Mice.* Healthy mice shall be used, all from a single strain and of the same sex, or an equal number of each sex in each group, with individual weights between 13 and 16 grams. A system of randomization shall be used to distribute the mice into the groups, with respect to shelf position and to determine the order of challenge. A group of at least 16 mice shall be used for each dilution of each vaccine. There shall be at least 4 groups consisting of no less than 10 mice each for control testing purposes, as required under paragraph (c) of this section.

(b) *Inoculation of vaccine.* (1) Serial dilutions, no greater than fivefold, of the vaccine to be tested and of the standard vaccine shall be made in saline (0.85 percent sodium chloride solution or phosphate-buffered saline). The mean

effective dose (ED_{50}) value shall be bracketed by the dilutions used. Each mouse in each group for inoculation shall be injected intraperitoneally with 0.5 milliliter of the appropriate dilution.

(2) The interval between inoculation of the vaccine and challenge shall be no less than 7 days nor more than 14 days. At least 87.5 percent of the mice in each group shall survive the period between vaccine inoculation and challenge and each mouse challenged shall appear healthy.

(c) *The challenge.* (1) The challenge culture of Strain Ty 2 of *S. typhi* for each test shall be taken from a batch of cultures maintained by a method, such as freeze-drying, that retains constancy of virulence.

(2) The challenge and virulence titration doses shall be prepared as follows: The bacteria shall be harvested from a 5- to 6-hour culture grown at 36°±1° C on a suitable agar medium that shall have been seeded from a 16- to 20-hour culture grown at 36°±1° C on a suitable agar medium, and the harvested bacteria then shall be uniformly suspended in saline or phosphate-buffered saline. The suspension, freed from agar particles and clumps of bacteria and adjusted to an opacity of 10 units, shall be diluted in saline or phosphate-buffered saline by tenfold increments. The suspensions for the challenge and virulence titration doses shall be put into a sterile gastric mucin preparation or other suitable virulence-enhancing preparation. The challenge suspension shall be prepared from whichever bacteria dilution provides about 1,000 colony forming units for a 0.5 milliliter challenge dose. The virulence titration suspensions shall be 10^1, 10^2, 10^3 dilutions, respectively, of the challenge suspension.

(3) Each mouse inoculated with vaccine shall be injected intraperitoneally with an 0.5 ml. dose of the challenge suspension. Each mouse in the four groups of control mice shall be injected intraperitoneally with an 0.5 ml. dose of the challenge suspension and its three dilutions, respectively. The challenge dose control mice shall be injected last. The interval between removal of the bacteria from the culture medium and the injection of the last mouse shall not exceed 2½ hours.

(d) *Recording the results.* The mice shall be observed daily for 3 days. A record shall be maintained of the number of mice that die. A record of the number of mice that survive shall be made at the end of the observation period.

(e) *Validity of the test.* The test is deemed valid if: (1) The ED_{50} of the vaccine under test and the standard vaccine is between the largest and smallest doses inoculated into the mice;

(2) The homogeneity of the dose response lines for both the vaccine under test and the standard vaccine is acceptable;

(3) A graded protective response is obtained in relation to the vaccine dilutions;

(4) The slopes of the dose response curves for the vaccine under test and the standard vaccine are shown to be parallel by an appropriate statistical method;

(5) The results of all dilutions are used to calculate the ED_{50} value of both the standard and test vaccine by a parallel line bioassay method or a statistically equivalent method;

(6) The challenge dose contains approximately 1,000 colony forming units; and

(7) The LD_{50} of the challenge dose contains no more than 20 colony forming units.

(f) *Repeat tests.* If the test does not meet the criteria prescribed in paragraph (e) of this section, repeat tests may be performed. The results of all tests shall be combined by geometric mean. Any test result established as invalid under §610.1 of this chapter may be disregarded. The determination that the vaccine meets the potency requirements shall be made from the results of not more than four valid tests.

(g) *Estimate of the potency.* The ED_{50} of each vaccine shall be calculated. The protective unit value per milliliter of the vaccine under test shall be calculated in terms of the unit value of the standard vaccine.

(h) *Potency requirements.* The results of at least two separate tests shall be included on the release protocol, required under §620.14(c)(2), that is submitted to the Center for Biologics

Evaluation and Research, Food and Drug Administration. The vaccine shall have a potency of 8.0 units per milliliter. This requirement shall be met only if the geometric mean potency for two tests is not less than 3.9 units per milliliter; or for three tests, not less than 4.4 units per milliliter; or for four tests, not less than 4.8 units per milliliter.

[38 FR 32064, Nov. 20, 1973, as amended at 48 FR 7167, Feb. 18, 1983; 49 FR 23834, June 8, 1984; 55 FR 11013, Mar. 26, 1990]

§ 620.14 General requirements.

(a) *Dose.* These standards are based on a human adult dose of 0.5 ml. for a single injection and a total immunizing dose of two injections of 0.5 ml. given at appropriate intervals.

(b) *Labeling.* In addition to the items required by other applicable labeling provisions of this subchapter, the package label shall state that the vaccine contains 8 units per milliliter.

(c) *Samples; protocols; official release.* For each lot of vaccine, the following material shall be submitted to the Director, Center for Biologics Evaluation and Research (HFB–1), 8800 Rockville Pike, Bethesda MD 20892.

(1) A sample of no less than 40 ml. of the product distributed in no less than four containers.

(2) A protocol that consists of a summary of the history of manufacture of each lot including all results of each test for which test results are requested by the Director, Center for Biologics Evaluation and Research.

(3) The product shall not be issued by the manufacturer until written notification of official release of each filling lot of dried vaccine and of each bulk lot of aqueous vaccine is received from the Director, Center for Biologics Evaluation and Research.

[38 FR 32064, Nov. 20, 1973, as amended at 42 FR 27582, May 31, 1977; 48 FR 7168, Feb. 18, 1983; 48 FR 11430, Mar. 18, 1983; 49 FR 23834, June 8, 1984; 51 FR 15610, Apr. 25, 1986; 55 FR 11013 and 11015, Mar. 26, 1990]

Subpart C—Anthrax Vaccine Adsorbed

§ 620.20 Anthrax Vaccine Adsorbed.

The proper name of this product shall be Anthrax Vaccine Adsorbed, which shall consist of an aqueous preparation of a fraction of *Bacillus anthracis* which contains the protective antigen adsorbed on aluminum hydroxide.

[38 FR 32064, Nov. 20, 1973, as amended at 50 FR 4137, Jan. 29, 1985]

§ 620.21 Production.

(a) *Strain of bacteria.* A nonencapsulated, nonproteolytic, avirulent strain of *Bacillus anthracis* shall be used in the manufacture of anthrax vaccine.

(b) *Medium.* A chemically defined medium shall be used for the propagation of *Bacillus anthracis* which has protective-antigen promoting properties that are no less effective than the protective-antigen promoting properties of the Puziss and Wright 1095 medium as set forth in U.S. Patent No. 3,208,909, issued September 28, 1965, which patent is hereby incorporated by reference and deemed published herein. U.S. Patent No. 3,208,909 has been assigned to the Federal Government and copies will be provided to persons affected by the provisions of this subchapter upon request to the Director, Center for Biologics Evaluation and Research, or to the appropriate Information Center Officer listed in 45 CFR, part 5. Copies also may be obtained upon request from the U.S. Patent Office, Washington, DC. The medium shall not contain ingredients known to be capable of producing allergenic effects in human subjects.

(c) *Propagation of bacteria.* The medium shall be inoculated with a 24-hour old vegetative culture seeded from a stock suspension of spores. The propagation culture, flushed with nitrogen, shall be incubated at 37° C.±1.0° C., agitated for approximately 27 hours, cooled to about 20° C., the pH adjusted to 8.0±0.1 and then filtered through a sterilizing filter(s) using nitrogen gas under pressure.